the degree to which this electrophoretic anomaly is related to the heredity of hemophilia, nor is it possible to predict from the electrophoretic patterns whether a woman will be a carrier of hemophilia or not. From the 7 members of hemophilic families, who are not bleeders themselves but have the electrophoretic anomaly, 3 are females. It is possible that these women will be carriers of the disease. A woman may be a carrier of hemophilia, however, without showing the electrophoretic anomaly. This follows from the observation that the mothers of the two families presented in this paper do not show the α_x-anomaly, although both are believed to be the carrier of hemophilia, the disease being transmitted in both families to male descendants.

Summary. 1. An electrophoretic anomaly, called α_x-globulin, has been found in the plasma of 14 hemophiliacs, *i.e.*, in all of the active bleeders studied. 2. The same anomaly also has been observed in 8 members of hemophilic families who are not active bleeders (over 50% of this category), and who are not afflicted with any of the other diseases in which this anomaly has been found. 3. The electrophoretic anomaly is seen in female as well as in male subjects unlike the bleeding manifestation of hemophilia, found only in males. 4. There is a definite relationship between the prothrombin utilization and the appearance of the α_x-globulin anomaly in the plasma of members of hemophilic families.

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Problems with Spontaneous Ectromelia (Mouse Pox) in a Virus Laboratory.* (20344)

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Since its discovery by Marchal(1), ectromelia virus, the causative agent of mouse-pox, has been recovered from several stocks of laboratory mice in Europe(2). Although mice have been reported in this country with a disease having similar signs, the virus has not been recovered from stock mice in the United States until recently when Trentin described an epizootic in the mouse colony of the Department of Anatomy at Yale(3). The conditions under which he isolated ectromelia virus are outlined in his paper. It was believed that the virus had been eliminated from the colony. However, we have recently observed a severe epizootic among our stock mice and have identified ectromelia virus as the causative agent. This report serves to indicate that the infection as late as February, 1953, was present among laboratory mice in the New Haven area.

The Outbreak. A number of non-specific deaths began to appear in the mouse colony of our laboratory in October and continued through the fall of 1952. During this period 200 pregnant mice arrived each week from 2 dealers. In addition, 200 to 300 were raised in New Haven each week for use when they reached the age of 4 weeks. For several months prior to the outbreak the newborn mice were being employed for isolating Coxsackie viruses from sewage and flies, and the adult mice chiefly for determining neutralizing antibodies against Lansing poliomyelitis virus. The total mouse population at any one time was a few thousand and rarely over 5000.

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TABLE I. Cross Hemagglutination Inhibition of Ectromelia and Vaccinia Viruses.

Serum source	Serum titer against 5 units of: Ectromelia virus	Vaccinia virus
Mice convalescent from spontaneous ectromelia infection	80	40
" 10 days after inj. of ectromelia virus	40	<20
Rabbit 242, immunized against vaccinia	80	80
" 243, " " "	80	80
" (McCrae) immunized against vaccinia	160	160

The incidence of non-specific deaths became so high that it was not possible to carry out neutralization tests in mice which had been in the laboratory for a few weeks. Skin lesions were not noted during the early part of the epizootic. In November occasional mice were seen with swollen or necrotic legs. An attempt to pass an infectious agent from such legs as well as from the livers of these mice met with failure. (As the recipient mice had been in the stock colony for at least a month, they might have been immune.) In January, the epizootic increased in virulence; the number of deaths in the colony increased to well over 50% of uninoculated animals and the appearance of swollen legs, which often became necrotic, increased markedly. The hind limbs were involved with much greater frequency than the forelimbs. Lesions of the skin, generally of the legs but also of the hairless areas of the body proper, were noted.

Passage of an agent from sick mice in early January was successful, both in 4- and 12-week-old mice, newly arrived in the laboratory. Intraperitoneal inoculation of 10% suspension of either liver or leg suspensions resulted in death of recipient mice, after an incubation period of about 6 days. Moribund animals often contained increased peritoneal fluid, the livers were pale (fatty) and sometimes white necrotic foci could be found. Inoculation into the foot pad resulted in 4 to 5 days in a localized swelling which was followed by necrosis of the distal portion of the limb. Death usually followed in a few days, although some animals recovered. The virus grew readily in the chorioallantoic membrane of the chick embryo yielding pocks such as described for ectromelia(4). Suspensions of infected membranes readily agglutinated red blood cells selected from chickens whose cells had been found to agglutinate in the presence of vaccinia virus. In all respects observed the agent behaved like ectromelia virus(2). As no known ectromelia virus was in the laboratory, the virus was identified antigenically by its cross reactions with vaccinia. These consisted of cross neutralization tests in chick embryos and of cross hemagglutination inhibition tests(5). As the virus was identified as that of ectromelia, it will be henceforth referred to by name.

Cross hemagglutination inhibition tests.‡ *Viruses.* First passage ectromelia virus on the chorioallantois and a rabbit testicular passage line of the Levaditi vaccinia strain were used. Two-fold dilutions of virus were made in 5% rabbit serum in saline (10 x 75 mm test tubes) and to 0.2 ml of each dilution, there was added 0.2 ml of 0.8% suspension of chicken cells (chicken No. 683). Readings made at 60 minutes gave a titer of 1:320 units for ectromelia and 1:640 for vaccinia. In the test 5 units of each virus were employed, *i.e.,* for ectromelia a dilution of 1:64 and for vaccinia, 1:128.

Sera. Five sera were employed: 1) sera taken from mice convalescent from the natural disease (1-22-53); 2) sera taken from first passage mice 10 days after inoculation in the foot pad (1-29-53); 3) 4) and 5) sera from rabbits immunized against the Levaditi vaccinia virus, No. 242 (3-27-47), No. 243 (3-27-47), and McCrae (1-20-53), respectively. Serum was diluted in saline serially in twofold steps, 0.2 ml of each dilution distributed in 2 test tubes. Ectromelia virus was added to one set, and vaccinia virus to the other, in each case 5 hemagglutinating units being present in 0.2 ml. After mixing, 0.2 ml

‡ Dr. J. F. McCrae kindly provided the chick cells, the vaccinia virus, and one of the vaccinia immune sera used in these tests.

of 0.8% suspension of washed chicken cells were added to each tube and readings made 60 minutes later. The results are shown in Table I.

The results show that the vaccinia sera inhibit hemagglutination by either virus to the same degree, the serum titers being 1:80 to 1:60. The ectromelia mouse sera yielded higher titers with the homologous virus. Thus one serum had a titer of 1:80 against ectromelia virus and only 1:40 against vaccinia. A less potent serum yielded a titer of 1:40 against ectromelia virus and failed to react with vaccinia virus at the lowest dilution tested, 1:20.

Cross neutralization tests in chick embryos. Chorioallantoic titrations were carried out in 12-day eggs using Burnet and Boake's modification of drawing the inoculum (0.1 ml) into the egg during the process of lowering the chorioallantoic membrane to form the base of the artificial air sac(5). Equal volumes of serial dilutions of virus and undiluted serum were mixed, held for one hour at room temperature, and 0.1 ml of each mixture inoculated into 4 eggs. Vaccinia-infected eggs were examined 48 hours later; ectromelia-infected eggs, 96 hours after inoculation.

The viruses in the test were first-passage foot pad suspension for ectromelia and the rabbit testicular suspension used above for vaccinia. Mice 10 days after inoculation of the foot pad suspension were the source of ectromelia serum, and a rabbit, No. 243 referred to above, immunized and bled in 1947 was the source of the vaccinia antiserum. The results are shown in Table II and indicate a cross relationship between the two viruses. The failure of the ectromelia serum to neutralize the homologous virus is probably an indication of the low antibody titer of this serum, for in the hemagglutination inhibition tests it had a titer of only 1:40 against the homologous virus and of less than 1:20 with vaccinia virus. The vaccinia antiserum neutralized both viruses.

TABLE II. Cross Neutralization Tests between Ectromelia and Vaccinia Viruses and Sera.

Serum	Virus titer	
	Ectromelia	Vaccinia
Ectromelia mice, 1-29-53	10^{-4}	10^{-6}
Vaccinia rabbit, No. 243, 3-27-47	10^{-2}	$<10^{-4}$
Saline control	10^{-4}	10^{-8}

Discussion. The virus isolated from the stock colony was identified as that of ectromelia by its biological properties and by its reciprocal relationship with vaccinia virus in serological reactions. Furthermore electron micrographs of thin sections of the chorioallantois of embryonated eggs infected with the local ectromelia strain have revealed virus particles of the pox group(8). The conditions under which the epizootic arose in our stock mice are unknown. Although the natural disease had appeared a year or so earlier in the stock mice of the Department of Anatomy, there is no known connection between mice or personnel handling mice in the two departments. However, as the virus is resistant to drying and subsequent storage at room temperature for 8 weeks(6), infected material may circulate through devious routes. One must also consider whether ectromelia virus was brought in from the outside. If this infection exists among wild mice(7), then it is conceivable that some specimens of sewage and flies which were inoculated into test mice may have contained the virus, although we believe that this possibility is remote. According to available information, the disease does not occur in the stock mice of either dealer from whom mice were obtained.

All mice in our laboratory were destroyed during the end of January and the beginning of February, 1953; all cages and bottles were sterilized; and the racks and room were washed down with 5% formalin. Virus harvests from mice infected with other agents during this period have also been destroyed, for ectromelia virus was undoubtedly present in some of the tissue suspensions stored in the frozen state. A new colony was brought in during February and to date the disease has not reappeared.

Summary. Trentin(3) has recently reported an outbreak of ectromelia (mouse pox) in his mouse colony at the Yale University School of Medicine. Another spontaneous epizootic in a different colony at the School is described in the present report. The virus

was isolated and was shown to be related to vaccinia virus by cross hemagglutination tests and cross neutralization tests. The infected colony as well as harvests of other viral agents prepared from mice in the colony have been destroyed.

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Intracellular Distribution of Digitoxin.* (20345)

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Recently we have demonstrated in various animals(1) that digitoxin readily leaves the blood stream to enter the various organs in quite significant amounts. However, the possible intracellular fate of digitoxin administered to the intact animal has never been determined because of the technical difficulties heretofore involved in such a study. Yet, in view of the uncertainty concerning both the site and mode of action of this glycoside, such information might be of considerable value. This is particularly so since the intracellular site of various enzymes now has been determined with a high degree of certainty.

On the introduction of the ultracentrifugal method for routinely isolating and obtaining intracellular components(2,3) and a microsensitive assay for the quantitative detection of digitoxin(4), it appeared possible that some information could be obtained concerning the intracellular fate of parenterally administered digitoxin. The results of a study using these technics are reported in this communication.

Methods. 1. *Separation and Collection of Intracellular Components.* The methods used, except for slight modifications, are those which have been described by Schneider and Hogeboom(3). The rats used were Long-Evans strain males, weighing 200 to 250 g. Rat liver was obtained five minutes after the intravenous injection of digitoxin (1 mcg. per gram of body weight) and rinsed in isotonic sucrose until the washings appeared free of gross blood. The liver was then forced through a tissue press into a beaker containing isotonic sucrose, the pulp weighed and sufficient sucrose solution added to prepare a 10% homogenate. Hearts were grossly dissected free of vessels and valves, weighed and minced with a scissor into the homogenizer containing sucrose. The homogenate was spun at 600 g for 10 minutes, the supernatant removed and the sediment washed, then respun again at the same speed and for the same period. The sediment obtained was identified by staining and microscopic study as the nuclear and debris fraction (Nw fraction)† The supernatant and washings were combined and spun at 5000 g for 10 minutes. The sediment was washed again and respun at 5000 g for 10 minutes. The sediment obtained was identified as the mitochondrial fraction (Mw2 fraction), the supernatant fluid identified as the fraction S_1 (no mitochondria were seen in this fluid) described by Schneider and Hogeboom(3). Similar fractions were obtained from the hearts of rats given the same quantity of digitoxin, except that an additional sedi-

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† The nomenclature used in labelling these fractions is that of Schneider and Hogeboom(3).